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Editor :

PROFESSOR T. DALLING, M.A., M.R.C.V.S.,
Professor of Animal Pathology and Director of The Institute of Animal Pathology, Cambridge University

Sub-Editor :

PROFESSOR J. McCUNN, M.R.C.V.S., M.R.C.S., L.R.C.P.,
Professor of Anatomy, The Royal Veterinary College

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Editorial

DISEASES IN DAIRY HERDS

FROM time to time we have written on the subject of diseases in dairy herds and have pointed out the losses associated with them. In the current number of THE VETERINARY JOURNAL we publish an article by J. J. MacGregor, Advisory Economist, in which he deals with the subject of "depreciation in the dairy herd." It will be observed that in 2,830 herds, embracing nearly 79,000 cows, the percentage of the animals disposed of for disease was 58.2. (See Table I, summarised from Economic Advisory Council's Committee on Cattle Diseases Report. Cmd. 4591, H.M.S.O., 1934.) Further, in Table IV, headed "Reasons for Disposal," of a total of 620 cows a total of 268 or 43.2 per cent. were disposed of for reasons of disease. These figures are interesting and are of considerable importance, in that they provide material for thought. In both tables analyses of the nature of the diseases are provided. The following are the figures: Sterility, 23.8 and 22.38 per cent.; abortion, 3.0 and 0.48 per cent.; udder disease, 7.7 and 4.68 per cent.; tuberculosis, Johne's disease and wasting, 9.7 and 4.68 per cent.; tuberculin tests, 3.6 and 5.16 per cent.; deaths and miscellaneous diseases, 10.4 and 5.65 per cent.

The incidence of sterility is high, but in all probability the figures in the tables do not represent the true state of disease of which sterility is the result. There is now ample evidence that the end result of a variety of diseases asso-

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ciated with the reproductive organs of cattle is sterility, though the presence of the diseases may not be recognised in their earlier stages. Diseases of the reproductive organs may fail to be detected for long periods, though the failure of the affected animal to breed may be recognised. In many cases a state of temporary infertility, varying in its duration, is known to exist. The interference with regular breeding is one of the causes of impaired milk production. It is impossible to assess the loss, even roughly, but it is admitted to be considerable. It is also known that early treatment of many of the diseases affecting the reproductive organs results in their early cure and the restoration of normal breeding. The causes of most of the conditions are now known. Research work, the results of which are capable of application by veterinary surgeons, has simplified the methods of diagnosis and treatment and further research will result in the acquiring of more information on the subject. There is no better example of the necessity of early diagnosis and treatment than in the control of infertility in cattle. It happens too often that the veterinary surgeon is not asked to attend till the advanced state of the disease has set up a condition of permanent sterility. It is encouraging that efforts are being made whereby early access of veterinary surgeons to breeding herds may be arranged and that early diagnosis and treatment of reproductive disorders may be undertaken.

The incidence of udder disease (7.7 and 4.68 per cent. respectively) evidently refers to mastitis of such a degree that the affected animals are regarded as economically useless for further milk production. It is known only too well that mastitis in its various forms and degrees is one of the more important causes of low milk yields. Again, in mastitis we have a disease, the presence of which may pass unnoticed till an acute attack discloses its existence. Further, it is known that the more chronic or subacute form of mastitis can be recognised by appropriate clinical tests. Here again, early and regular examinations by a veterinary surgeon go a long way to assist in the control of the disease.

These are but two examples of the value of regular examinations of dairy cattle by veterinary surgeons in increasing the milk supply of the country. There seems little doubt that any system whereby such a service can be organised, even on a small scale as a beginning, should be followed by good results: any scheme, however small, provided its results demonstrate its value, is bound to be taken up to an ever-increasing extent and to become established. Veterinary surgeons can do much to impress their clients with the necessity for early diagnosis and treatment. It is also of importance that the best methods are used in such treatment and it is opportune to point out at this time that veterinary surgeons should take every opportunity of acquiring the necessary knowledge of the techniques and methods of treatment which are recognised as producing the most satisfactory results.

General Articles

CHEMICAL CHANGES OCCURRING DURING THE GROWTH OF VARIOUS ACID-FAST BACTERIA IN SYNTHETIC MEDIA

By A. EDEN and H. H. GREEN

(Veterinary Laboratory, Ministry of Agriculture and Fisheries, New Haw, Weybridge)

THE use of synthetic media of known chemical composition for the growth of various acid-fast bacteria in place of the chemically ill-defined and complex glycerinated broth of Koch has been responsible for very considerable advances in our knowledge of the bacteria and their products. Preparation of tuberculins of greater specificity has thus been rendered possible for the diagnosis of tuberculosis infections in man and in animals; the active protein fractions have been isolated, mainly as a result of the work of Long and Seibert, and this has given rise to the important "Purified Protein Derivatives"; and Anderson and his colleagues have been able to make extensive studies on the chemistry of the bacteria themselves. The adoption in this country of the "synthetic medium tuberculin" of the American Bureau of Animal Industry for official tests in cattle has rendered desirable further knowledge of the chemical changes which occur during the growth of the tubercle organisms on the synthetic medium employed. At the same time it was considered equally instructive to study the metabolism of other species and strains of *M. tuberculosis* and of certain other acid-fast organisms such as the economically important *M. paratuberculosis* of Johne and the non-pathogenic *M. phlei*.

It is not proposed to discuss in detail the already extensive literature on the subject. The position up to 1932 is adequately covered by Wells and Long in "The Chemistry of Tuberculosis." Long in 1919 had reported that human and bovine types of tubercle bacilli produce ammonia when grown on a synthetic medium containing glycerine, acetamide or asparagine as the source of nitrogen. Johnson *et al.* (1928, 1929 and 1930) described in great detail the effect of the growth of human, bovine and avian types of tubercle bacilli on Long's medium which contained principally asparagine, ammonium citrate and glycerol, with small quantities of various inorganic salts. They studied in particular the rate of growth, change of pH, ammonia production and phosphate utilisation. The ability of human and avian types of tubercle bacilli

to utilise glycerol suggested to Dorset and Henley (1934) the desirability of increasing the amount of growth by raising the glycerol content to 10 per cent. and simultaneously increasing the asparagine from 0.5 to 1.2 per cent. The present synthetic medium recommended by the B.A.I. contains 1.4 per cent. asparagine. We have found *M. phlei* to grow equally well on this medium but as is well known the bovine type of tubercle bacilli cannot tolerate a high glycerol concentration and the modified Sauton medium of Watson and Reid (1935) has been used in the present studies. Asparagine seems to be one of the best sources of nitrogen although Henley and leDuc (1930) stated that ammonium malate could be equally well employed. Henley and leDuc (1939) made extensive studies on media with various nitrogen sources and varying amounts of glycerol, employing the human PN strain. In the present studies we have employed essentially the analytical methods suggested by Henley and leDuc, with certain modifications. Attention has been devoted almost exclusively to the gross metabolism of the organic constituents of the media employed, the intermediary metabolism and mineral metabolism awaiting possible investigation in the future.

Experimental Methods and Material

The acid-fast bacteria studied included three strains of the human type as used by the B.A.I. in the manufacture of tuberculin in the U.S.A.; one strain each of the bovine and avian types; one strain of the non-pathogenic *M. phlei* and two strains of *M. paratuberculosis*. The synthetic media employed were the B.A.I. modification of Dorset and Henley's medium and the modified Sauton medium of Watson and Reid (1935), of the following composition:—

B.A.I. medium		*Watson & Reid*	
Asparagine	14.0 g.	Asparagine	5.0 g.
K_2HPO_4. $3H_2O$ ...	1.8 g.	$MgSO_4$. $7H_2O$ ...	1.0 g.
$MgSO_4$. $7H_2O$ ...	1.5 g.	KH_2PO_4	2.0 g.
Sodium Citrate ...	0.9 g.	Ammonium Citrate ...	2.0 g.
Ferric Citrate	0.3 g.	NaCl	2.0 g.
Dextrose	10.0 g.	Ferric Ammonium Citrate	0.075 g.
Glycerine	100.0 g.	Dextrose	10.0 g.
Water q.s.	1000.0 ml.	Glycerine	100.0 g.
pH 6.8-7.0 ...	... No adjustment	Water q.s.	1000.0 ml.
		Adjusted with ammonia to pH 7.2 to 7.4 except for the Johne's strains.	

100 ml. portions were pipetted into 300 ml. Erlenmeyer flasks and sterilised at 15 lbs. pressure for seven minutes. The cultures were kindly supplied through Mr. J. Reid, of the Tuberculin Department of this laboratory.

The flasks were inoculated and grown at 37-37.5 deg. Cent. At known intervals one flask was removed and sterilised in streaming steam for three hours. The bacterial bodies were filtered off through a weighed paper and washed with hot water, dried overnight at 100-105 deg. Cent. and reweighed. The filtrate and washings were made up to 200 ml. and except for one human and the avian strain the pH was immediately determined electrometrically. The following was the scheme of analysis :—

Bacterial N : Kjeldahl method on the washed bacterial bodies.

Filtrate total N : Kjeldahl method on 20 ml. portions.

Ammonia-N : 40 ml. portions were diluted with 200 ml. water, 1 g. MgO added and the ammonia distilled off into standard acid.

Amido-N : The residue from the ammonia distillation was diluted with 150 ml. water, 30 ml. 50 per cent. NaOH added and distillation continued into further portions of standard acid.

Protein N : A 50 ml. aliquot part was treated with an equal volume of 20 per cent. trichloracetic acid and left to stand overnight. The precipitate was centrifuged and washed three times with 50 ml. two per cent. trichloracetic acid. The N in the precipitate was determined by the Kjeldahl method.

Glycerol : 4 ml. portions were oxidised by 25 ml. 1.52 N $K_2Cr_2O_7$ and 20 ml. conc. H_2SO_4 in a boiling water bath for 20 minutes. After diluting to 100 ml., 5 ml. portions were treated with KI and the liberated I_2 titrated with N/10 thiosulphate. All the oxidisable organic matter was recorded as glycerol.

Dextrose : Micromethod of Hagedorn and Jensen.

Corrected glycerol : Since dextrose is also oxidised by dichromate the glycerol equivalent of the sugar was subtracted, 1 g. dextrose registering as 0.89 g. " glycerol."

Calculations : All figures were referred to the original 100 ml. medium.

Original N in the medium minus (bacterial N + filtrate N) = Lost N.
Filtrate N plus Lost N = Corrected filtrate N.
NH_3-N plus Lost N = Corrected NH_3-N.
Filtrate N as determined minus (protein N + NH_3-N as determined + amido-N) = Amino-N.

Some of these methods, e.g., the recording of amido-N by distillation with strong soda and of amino-N by difference, are obviously only broad approximations and it is recognised that they may include unspecified nitrogenous products of bacterial metabolism, but they were regarded as adequate for the primary orientation in view. Nucleic acid and polysaccharide associated with the protein fraction, or free in the filtrate, were not determined in these preliminary studies, but may be included in probable later work after adaptation of theoretically available methods. Dextrose as determined by the Hagedorn

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TABLE I.

Chemical Changes Associated with the Growth of Three Strains of Human Type Tubercle Bacillus on B.A.I. Synthetic Medium. Analytical Data in g. per 100 ml. original medium.

Constituent	Strain DF Human										Strain C Human										Strain DF Human									
	Duration of Growth (days)										Duration of Growth (days)										Duration of Growth (days)									
	Initial level	12	19	26	33	40	47	54	61	68	75	Initial level	10	24	31	38	45	52	59	66	73	Initial level	12	19	26	33	40	47	54	61	68	75
Weight of Bacteria		0.141	0.695	0.797	1.019	1.530	1.542	1.589	1.900	1.536	1.508		0.055	0.616	0.981	1.303	1.616	1.568	1.307	1.476	1.297		0.105	0.443	0.714	0.907	1.338	1.694	1.721	1.638	1.557	1.560
Bacterial N		0.018	0.084	0.076	0.088	0.134	0.114	0.823	0.169	0.118	0.126		0.007	0.077	0.081	0.118	0.130	0.197	0.128	0.139	0.115		0.009	0.056	0.065	0.081	0.115	0.120	0.126	0.127	0.122	0.130
Filtrate N as determined		0.252	0.195	0.181	0.197	0.186	0.115	0.099	0.089	0.107	0.105		0.248	0.152	0.126	0.123	0.089	0.090	0.116	0.111	0.121		0.226	0.186	0.158	0.126	0.108	0.076	0.079	0.094	0.100	0.101
" " corrected	0.288	0.253	0.211	0.171	0.159	0.111	0.121	0.122	0.078	0.127	0.119	0.260	0.253	0.186	0.169	0.148	0.110	0.125	0.126	0.121	0.143	0.248	0.208	0.170	0.153	0.132	0.096	0.089	0.108	0.113	0.115	
Lost N		0.001	0.016	0.020	0.022	0.023	0.018	0.026	0.007	0.020	0.018		0.006	0.021	0.013	0.017	0.021	0.024	0.020	0.020	0.024		0.009	0.019	0.023	0.025	0.024	0.017	0.010	0.014	0.015	0.014
Ammonia N as determined	0.034	0.029	0.029	0.027	0.028	0.040	0.049	0.026	0.029	0.029	0.033	0.046	0.027	0.026	0.026	0.027	0.027	0.028	0.026	0.031	0.025	0.004	0.024	0.028	0.021	0.028	0.031	0.029	0.029	0.029	0.029	0.031
" N corrected		0.050	0.036	0.107	0.036	0.053	0.067	0.051	0.015	0.039	0.049		0.023	0.107	0.079	0.054	0.048	0.051	0.029	0.051	0.039		0.024	0.107	0.113	0.108	0.073	0.046	0.029	0.043	0.046	0.048
Amido-N	0.121	0.036	0.041	0.006	0.007	0.009	0.008	0.028	0.008	0.006	0.008	0.126	0.111	0.009	0.007	0.009	0.020	0.007	0.009	0.009	0.007	0.121	0.100	0.020	0.008	0.006	0.008	0.007	0.006	0.006	0.007	0.006
Amino-N	0.120	0.108	0.071	0.050	0.043	0.025	0.039	0.022	0.020	0.035	0.044	0.130	0.120	0.029	0.073	0.027	0.027	0.045	0.025	0.028	0.028	0.120	0.110	0.026	0.026	0.041	0.031	0.028	0.023	0.028	0.040	0.047
Protein N		0.009	0.004	0.009	0.012	0.017	0.020	0.025	0.022	0.027	0.020		0.000	0.018	0.010	0.015	0.017	0.019	0.020	0.020	0.023		0.008	0.004	0.003	0.013	0.016	0.016	0.018	0.018	0.021	0.017
"Glycerol" as determined	15.84	10.10	9.48	7.67	5.33	3.48	3.06	1.71	0.46	0.96	0.63	10.90	10.75	7.01	6.48	4.45	1.36	0.99	0.82	0.89	0.65	10.84	10.35	9.34	6.05	5.61	4.31	1.38	0.55	0.90	0.62	0.65
Dextrose	0.86	0.46	0.34	0.08	0.07	0.06	0.05	0.04	0.04	0.05	0.05	0.84	0.30	0.20	0.18	0.19	0.07	0.07	0.08	0.07	0.07	0.86	0.47	0.21	0.10	0.08	0.05	0.05	0.04	0.05	0.05	0.05
Corrected "glycerol"	10.00	9.69	9.27	7.60	5.27	3.57	3.01	1.67	0.42	0.52	0.58	10.05	10.01	6.85	6.27	4.54	1.30	0.92	0.82	0.64	0.60	10. 0	9.81	9.15	7.94	6.54	4.35	1.34	0.49	0.46	0.58	0.58
pH	6.9	7.6	8.6	9.1	9.2	9.3	9.2	9.2	9.2	9.2	9.2											6.9	7.2	8.7	9.1	9.2	9.3	9.2	9.0	8.8	8.7	8.4

TABLE III.

Chemical Changes Associated with the Growth of Bovine Type Tubercle Bacillus and two strains of M. paratuberculosis on Watson and Reid's Synthetic Medium. Analytical Data in g. per 100 ml. original medium.

Constituent	Bovine Strain A.B.E.											M. Paratuberculosis Strain XII and V.										M. Paratuberculosis Strain 286.									
	Duration of Growth (days)											Duration of Growth (days)										Duration of Growth (days)									
	Initial Level	10	17	22	29	36	43	50	57	64	71	78	Initial Level	10	17	24	31	38	46	54	61	68	Initial Level	12	19	26	33	40	48	54	63	70
Weight of bacteria.		0.215	0.472	0.564	0.652	0.811	0.894	1.004	1.057	1.106	1.188	1.174		0.476	1.092	1.216	1.194	1.106	1.060	1.012	1.085	0.904		0.018	0.056	0.286	0.448	0.513	0.906	1.168	1.088	0.907
Bacterial N.		0.029	0.043	0.053	0.057	0.073	0.067	0.064	0.064	0.069	0.108	0.105		0.037	0.090	0.096	0.089	0.089	0.089	0.089	0.087	0.085		0.008	0.005	0.021	0.038	0.039	0.080	0.083	0.084	0.081
Filtrate N as determined	0.143	0.106	0.090	0.079	0.071	0.059	0.047	0.040	0.041	0.036	0.040	0.054	0.136	0.091	0.054	0.053	0.051	0.053	0.052	0.039	0.039	0.035	0.136	0.135	0.119	0.100	0.080	0.068	0.047	0.048	0.048	
" " corrected		0.121	1.100	0.080	0.086	0.070	0.056	0.049	0.049	0.044	0.041	0.056		0.091	0.058	0.054	0.056	0.056	0.056	0.056	0.039	0.041		0.136	0.121	0.109	0.084	0.069	0.056	0.043	0.048	0.048	
Lost N.		0.015	0.010	0.011	0.015	0.011	0.009	0.009	0.008	0.003	0.001	0.008		0.000	0.008	0.008	0.008	0.008	0.004	0.003	0.000	0.003		0.000	0.008	0.008	0.004	0.007	0.003	0.001	0.000	0.008	
Nitrogen as determined.	0.051	0.076	0.067	0.061	0.061	0.037	0.034	0.016	0.016	0.013	0.009	0.008	0.035	0.046	0.009	0.008	0.005	0.001	0.009	0.005	0.004	0.004	0.035	0.037	0.048	0.041	0.047	0.040	0.039	0.035	0.008	0.008
" " " corrected.		0.026	0.017	0.019	0.026	0.018	0.015	0.015	0.014	0.017	0.010	0.008		0.046	0.011	0.007	0.008	0.007	0.008	0.007	0.004	0.007		0.037	0.044	0.044	0.031	0.047	0.038	0.035	0.008	0.008
Amido-N.	0.045	0.014	0.004	0.005	0.004	0.005	0.005	0.005	0.004	0.004	0.004	0.005	0.045	0.008	0.008	0.003	0.005	0.005	0.005	0.005	0.004	0.005	0.035	0.043	0.036	0.008	0.004	0.008	0.008	0.005	0.010	0.009
Amino-N.	0.047	0.017	0.011	0.008	0.010	0.010	0.010	0.010	0.014	0.014	0.018	0.021	0.054	0.060	0.018	0.021	0.025	0.028	0.025	0.029	0.024	0.021	0.048	0.044	0.050	0.034	0.038	0.024	0.026	0.028	0.026	0.024
Protein-N.		0.001	0.006	0.007	0.008	0.007	0.008	0.009	0.009	0.009	0.006	0.006		0.004	0.009	0.003	0.003	0.003	0.006	0.006	0.007	0.010		0.000	0.008	0.004	0.004	0.008	0.004	0.010	0.008	0.004
Glycerol as determined	6.58	6.19	5.40	4.87	4.01	3.72	2.97	2.86	2.43	2.28	1.80	1.66	6.83	4.94	2.78	2.30	1.72	1.48	1.41	0.46	0.58	0.40	6.58	6.51	4.35	3.19	2.36	2.32	1.97	0.86	0.43	0.43
Dextrose	0.89	0.34	0.11	0.07	0.07	0.06	0.06	0.06	0.06	0.06	0.07	0.09	0.89	0.30	0.10	0.07	0.06	0.06	0.10	0.06	0.05	0.05	0.85	0.60	0.14	0.40	0.29	0.26	0.18	0.13	0.11	0.13
Glycerol as corrected	5.80	5.71	5.50	4.61	3.95	3.66	2.93	2.83	2.39	2.25	1.85	1.61	6.08	4.60	2.64	2.26	1.65	1.40	1.35	0.41	0.49	0.36	6.08	5.96	5.07	4.69	3.13	2.99	1.81	0.90	0.35	0.32
pH	6.8	8.7	8.9	8.8	7.8	7.1	8.1	8.9	8.8	8.7	8.9	8.9	8.7	8.7	8.7	8.8	8.8	8.9	8.7	8.3	8.1	8.8	8.7	8.9	8.4	7.1	7.8	7.1	8.0	8.7	8.5	8.1

and Jensen method presumably includes small quantities of reducing bodies formed during bacterial growth but this does not affect the broad conclusions drawn from the data.

I. Bacteria grown on B.A.I. medium

Human Type.

Strains C, PN and DT as employed in the manufacture of B.A.I. tuberculin were used in these studies, and the full analytical results are recorded in Table I. Maximum weight was reached in just over six weeks with strains C and PN, in eight weeks with DT. In no case did this maximum attain the dry weight of 2 g. as Dorset (1934) suggested. Most growths showed a tendency to sink after about the 60th day and there was a decrease in weight presumably due to autolysis. The bacterial N comprised roughly 9.3 per cent. of the total weight although the figure was by no means constant. As growth proceeded the pH values increased and in all cases the medium was distinctly alkaline at the termination of the experiment. This was accounted for by the production of ammonia, especially during the third and fifth weeks, the period of most active early bacterial growth, and over 40 per cent. of the initial total nitrogen of the medium was present as ammonia nitrogen. During this period the most rapid disappearance of the glycerol occurred, indicating that metabolic products of the glycerol were being elaborated into the somatic substance of the bacteria as well as being used for respiration. Also at this time the amide nitrogen rapidly fell to a low level, a characteristic feature of the metabolism. The amino-nitrogen fell to below half the initial level and then gradually increased, but since this estimation was a " difference determination " it may be that part of the amino-N was converted into some other undetermined form of nitrogen, and in the absence of further analytical information it is perhaps unwise to speculate on its possible significance. The rapid disappearance of the dextrose over the first month of growth suggests that the sugar is utilised initially in preference to glycerol as a source of carbon.

From the aspect of tuberculin production the protein nitrogen fraction is the most important and from Table I it is seen that there is a slow, steady increase in this fraction throughout the course of growth. The work of Seibert suggests that the method of protein determination by precipitation at 10 per cent. concentration of trichloracetic acid also brings down some nucleic acid, presumably as " protein nucleate," but what the quantity is and how far it may be removed in the further washings with more dilute trichloracetic acid cannot at present be stated with precision. Analyses of the " Purified Protein Derivative " (not reported here) suggest that the amount of nucleic acid is not more than 10 per cent. and the general conclusions drawn from a study of the protein nitrogen figures are not invalidated by the presence of small amounts of nucleic acid nitrogen.

Despite the decrease in bacterial nitrogen and in weight of bacteria due to autolysis after maximum growth is reached, no corresponding increase in the

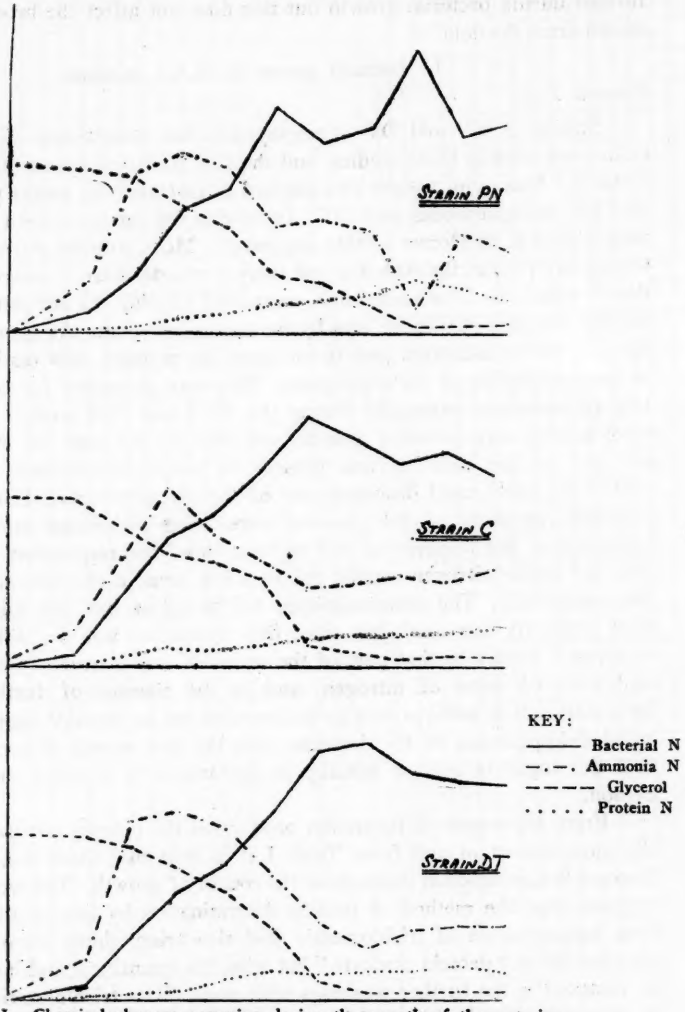

FIG. 1.—Chemical changes occurring during the growth of three strains of human type tubercle bacillus on B.A.I. synthetic medium.

protein nitrogen was shown in this series of experiments. The PN strain at the 61st day showed the maximum bacterial weight and the maximum protein

nitrogen figure recorded in the present series, but, on the whole, by the end of the 8th or 9th week the amount of soluble protein nitrogen recorded with all three strains was about 20 mg. per 100 ml., corresponding to about 0.14 g. of the active protein principle. It may be mentioned, incidentally, that these strains were chosen by the B.A.I. for their capacity to produce tuberculins of high potency.

The main features of the growth changes in respect of bacterial, ammonia and protein nitrogen, and glycerol fractions are shown diagrammatically in Figure I. Broadly speaking, the general characteristics of the growth are in same for all three human strains.

Avian Type.

In contrast with the slower growth but extensive production of "tuberculin protein" of the human type, an avian strain D4 was found to grow very rapidly and the cultures began to sink by about the fifth week. The full analytical details are given in Table II. Growth was exceptionally heavy and nearly 2.7 g. dry bacterial weight were obtained after 4½ weeks. The amount of nitrogen in the avian bacterial bodies was only about half that found in the human types, and the production of ammonia was also only half that of the human type at the most active phase of growth. The heaviness of the growth was attained by the rapid utilisation of the two carbon sources, dextrose and glycerol. The rate of utilisation of amido- and amino-nitrogen was comparatively slow and it was not until the cultures had attained maximum growth that the amido-nitrogen fell to the low level characteristic of the human type. The production of tuberculo-protein was only from one-third to one-half that of the human types in a corresponding time. The organisms appear to be more active in building up their somatic structure than in elaboration of the water-soluble active principle. We have also made studies, not in the same detail, of six other avian strains in respect of their capacity for active protein production and in each case protein nitrogen values of the same order as reported for D4 were found. Some of these strains did not sink as rapidly as the D4 strain but all produced a heavy somatic growth, of low bacterial N content, and protein N values lying between 0.005 and 0.010 g. per 100 ml., and it would appear that the results given here for the D4 strain are typical of those for the avian type of tubercle bacillus generally. The more important aspects of the analyses in Table II are shown graphically in Figure II.

M. phlei.

As a representative of the non-pathogenic acid-fast bacilli the timothy grass organism was next studied in the same way as with the preceding types. The results are given in Table II and the more important features shown graphically in Figure II. Growth was more rapid than for the human tubercle bacillus and maximum weight was reached in less than a month. The per-

centage of nitrogen in the bacterial mass was of the same order as for the human type. The growth began to sink by about the seventh week. pH determinations showed again a progressive alkalinisation of the culture media, the increase being particularly marked after maximum growth was reached. Ammonia production was again a prominent feature, although the maximum

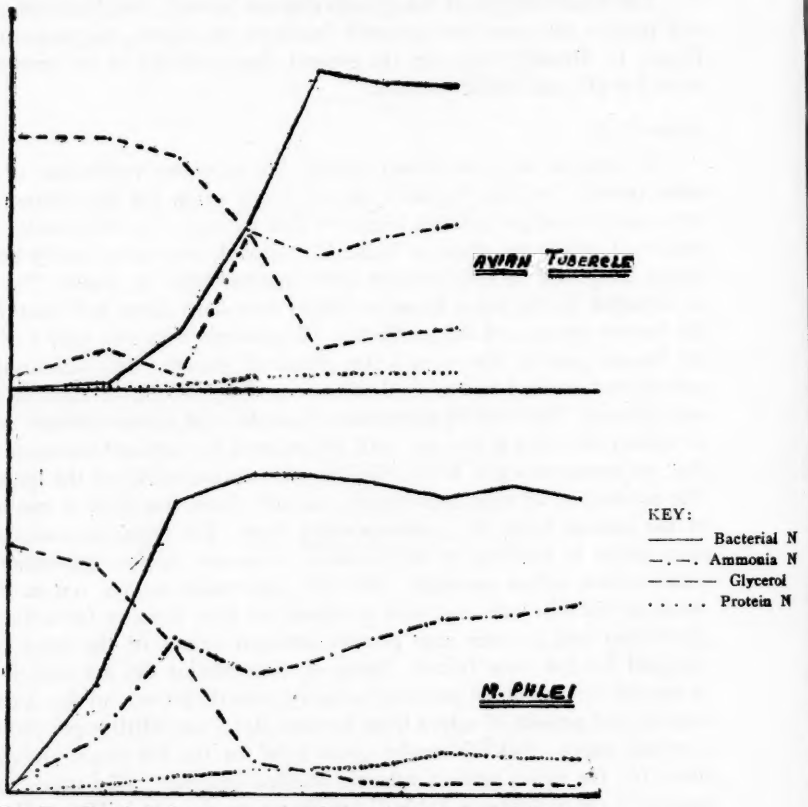

FIG. II.—Chemical changes occurring during the growth of Avian type tubercle bacillus and Timothy bacillus on B.A.I. synthetic medium.

values did not attain the peak shown by the human strains. The glycerol was rapidly exhausted, so that after a month the effective concentration was practically negligible. The removal of this potential source of organic acid production probably accounts for the increase in pH after this time as ammonia formation, possibly derived by autolysis of the bacteria, continued to occur. No significant changes occurred in the amino and amido-

nitrogen fractions after the growth peak was reached; the latter fraction again practically disappeared from the medium in the early stages of growth. Following the peak the protein nitrogen showed a 50-70 per cent. increase in yield accompanied by decrease in bacterial nitrogen and increase in ammonia formation. These findings may be the result of autolysis of the cells. At the moment it is impossible to state whether the increase in protein, occurring as a result of autolysis, implies a corresponding increase in its capacity to elicit skin reactions in suitably sensitised guinea pigs; this point must await future experimentation.

II. Bacteria grown on Watson and Reid's medium

Bovine Type.

As was expected, the use of the modified Sauton medium did not lead to such heavy growths as the B.A.I. medium containing larger amounts of glycerol and asparagine. The bovine type of tubercle bacillus made good growth on the medium but maximum weight was not reached until the cultures began to sink at the 11th week, although the growth increase after the 7th week was slight. The bacterial nitrogen content bore about the same relation to the total growth weight as was found for the human type. The pH values, after slightly increasing in the early stages of growth, gradually decreased and the medium became acid, finishing at pH 5.0. These values are reflected in the figures for ammonia nitrogen, which after an initial increase gradually diminished and then practically disappeared. The amido-nitrogen practically disappeared at an early phase of the growth, and a similar picture was seen for the amino-nitrogen which, however, gradually rose again towards the end. As stated earlier, in the absence of more detailed analyses it is impossible to say how far this represents some undetermined nitrogen fraction. The protein nitrogen attained its maximum within the first three weeks of growth and within the limits of individual and experimental error remained stationary thereafter at a level of only one-third that of the human strains. The dextrose was, as usual, rapidly used up but the glycerol values fell gradually and slowly, although less than 75 per cent. of the initial quantity present had been utilised by the end of 11 weeks of growth. An attempt was made to grow this strain on a medium containing twice the stated amount of asparagine to discover if this was a limiting factor but the growth was poor and the utilisation of the glycerol very slow, although a protein nitrogen figure of the same order as found in the initial growth was recorded. The full analytical data are given in Table III and a graphical representation of the more important features of the growth is shown in Figure III.

M. paratuberculosis.

Two strains of Johne's bacillus, a fast mixed strain III and V, and a slower growing strain 286, able to grow on synthetic media without the

TABLE II.

Chemical Changes Associated with the Growth of Avian Type Tubercle Bacillus, and Timothy bacillus on B A.I. synthetic medium.

Analytical data in g. per 100 ml. medium.

	Avian Strain D.4.							M. Phlei. Strain							
CONSTITUENT.	Initial	Duration of Growth (days)						Initial	Duration of Growth (days)						
	Level	10	17	24	31	38	45	Level	9	17	25	30	37	44	51	58
Weight of bacteria		0.042	0.302	1.718	2.667	2.201	2.103		0.448	1.265	1.620	1.389	1.334	1.101	1.063	1.056
Bacterial N.		0.003	0.023	0.067	0.128	0.123	0.122		0.044	0.118	0.129	0.129	0.126	0.102	0.097	0.095
Filtrate N as determined	0.259	0.248	0.236	0.164	0.116	0.125	0.126	0.253	0.208	0.123	0.114	0.115	0.110	0.120	0.125	0.120
" " corrected		0.256	0.236	0.192	0.131	0.136	0.137		0.209	0.135	0.124	0.124	0.126	0.151	0.156	0.158
Lost N.		0.008	0.000	0.038	0.015	0.011	0.011		0.001	0.012	0.010	0.009	0.016	0.031	0.031	0.038
NH_3 - N as determined	0.005	0.008	0.006	0.024	0.039	0.061	0.053	0.003	0.021	0.051	0.039	0.044	0.047	0.046	0.040	0.040
" " corrected		0.016	0.006	0.062	0.054	0.062	0.067		0.022	0.063	0.049	0.053	0.063	0.071	0.071	0.078
Amido - N	0.127	0.120	0.111	0.056	0.011	0.004	0.007	0.122	0.084	0.017	0.010	0.008	0.007	0.007	0.007	0.007
Amino - N	0.127	0.119	0.117	0.070	0.060	0.063	0.059	0.128	0.100	0.048	0.055	0.053	0.045	0.050	0.062	0.058
Protein - N.		0.001	0.002	0.005	0.006	0.007	0.007		0.003	0.007	0.010	0.010	0.012	0.017	0.016	0.015
Glycerol as determined	10.92	10.86	10.13	6.77	1.68	2.27	2.60	10.48	9.48	5.92	1.38	0.49	0.62	0.66	0.53	0.63
Dextrose	0.96	0.91	0.90	0.44	0.04	0.13	0.13	0.93	0.34	0.03	0.07	0.07	0.06	0.06	0.05	0.05
Glycerol as corrected	10.07	10.05	9.33	6.38	1.65	2.15	2.48	9.60	9.18	5.89	1.32	0.43	0.47	0.52	0.49	0.49
pH.								6.8	7.6	8.9	7.8	8.1	9.2	9.4	9.1	9.0

addition of *M. phlei* extracts were obtained by Mr. T. M. Doyle, Deputy-Director of this laboratory, from Canada, kindly supplied by Dr. Watson. The faster strain III and V attained its maximum growth of 1.12 g. within a month of inoculation. The rapid utilisation of the dextrose is again a feature, as is seen from the full protocols in Table III, and the glycerol values fell rapidly at first and then more slowly after maximum growth was reached,

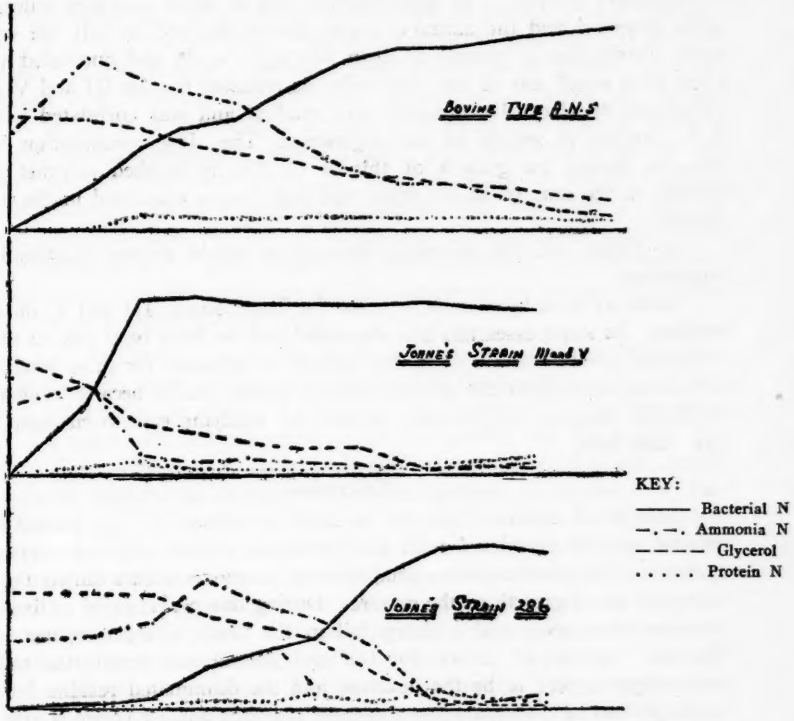

FIG. III.—Chemical changes occurring during the growth of bovine type tubercle bacillus and two strains of Johnes' bacillus on Watson and Reid's synthetic medium.

the glycerol probably being used up by continued respiration of the organisms. A slight increase in ammonia production and pH values occurred initially, but the pH figures then tended to fall and the ammonia gradually and almost completely disappeared. The amido-nitrogen also rapidly disappeared but the amino-nitrogen, after dropping to less than half the initial figure, remained practically constant. Protein nitrogen reached a maximum after 17 days and

variations were found between individual figures from week to week, but the general level was of the same order as reported for the bovine strain.

The slower growing strain required eight weeks to attain maximum growth. The pH values at first increased from the initial 5.7 which appears to be the optimum for the growth of these strains (Watson, 1935) and then fell to below this level towards the end of growth. The ammonia nitrogen figures at first increased but then fell to a very low level after eight weeks, the point of maximum growth. The characteristic fall in amido-nitrogen values was again observed and the amino-nitrogen figures dropped to half the original level. Production of protein nitrogen was again slight and fluctuated around a low level which was of the same order as reported for the III and V strain. Utilisation of glycerol was again very gradual and was correlated with the slow increase in weight of the organisms. The slow consumption of the dextrose during the growth of this strain was in marked contrast to the findings in the case of all the other acid-fast strains examined in the present studies.

In Figure III the principal findings are again shown graphically for comparison.

Attempts have been made to grow the faster strain, III and V, on B.A.I. medium. In some cases this has succeeded and we have been able to produce successive generations although little growth is apparent for three weeks. By continuous sub-culture the growth became weaker and it became evident that the B.A.I. medium was not very suitable for studying growth changes of the type made here.

Discussion

Growth of acid-fast bacteria on B.A.I. medium is accompanied by an increase in pH associated with the formation of considerable amounts of ammonia. The most extensive production of ammonia occurs during the early active phases of growth of the culture. During this period rapid utilisation of dextrose takes place and a sharp fall in the amido-nitrogen values occurs. The main sources of carbon for bacterial growth and respiration over the early stages appear to be the dextrose and the deaminated residue from the amide portion of the asparagine molecule, although some glycerol is also used. As growth proceeds more extensive splitting of the glycerol occurs, though the intermediate metabolic products have not been isolated, and the glycerol acts both as a source of energy for respiration and a source of carbon for the rapidly increasing bacterial mass. The ammonia derived from the earlier splitting of the asparagine diminishes by utilisation for building up into the somatic substance of the bacteria as the maximum growth peak is attained. In the case of the timothy bacillus the ammonia production reached an early peak, began to fall slightly, and then increased again as autolysis proceeded.

The reaction of the medium, always on the alkaline side of neutrality, is

dependent upon various factors such as the rate of utilisation of the asparagine and glycerol and the metabolism of the phosphates. Withdrawal of ammonia leaves an acid medium but utilisation of the acid portion of the deaminated asparagine residue without corresponding use of the ammonia tends towards an alkaline reaction. Intermediary products of oxidation of glycerol are presumably also acid.

On the other hand, the growth of bovine type tubercle bacillus and of *M. paratuberculosis* on the modified Sauton medium always tended towards lowering the pH of the medium. These organisms possess cultural characteristics such that they cannot tolerate a high proportion of glycerol. In the initial stages of growth the culture medium became more alkaline, due to the production of ammonia, probably as a result of splitting of the amide portion of the asparagine molecule, but with increasing growth the ammonia content diminished and the medium became acid. The two strains of Johne's bacillus appear to grow best on an initially acid reaction. The utilisation of amido- and amino-nitrogen followed the same general characteristics as for the organisms growing on B.A.I. medium. The slow utilisation of the glycerol was in marked contrast to the rapid use of this compound by human and avian types of tubercle bacilli and by *M. phlei.*

The most interesting feature lay in the production of water-soluble non-coagulable protein, since it is on this factor that the potential capacity for producing tuberculins depends. Apart from the question of specificity the activity of a tuberculin depends upon its protein content and the greater the capacity of various strains of acid-fast bacteria to produce the water-soluble non-coagulable protein the higher will be the potency of products of the tuberculin and johnin class made from it. Obviously, then, if by selection the highest yielding strains of each type can be secured the potency of the resulting product will be enhanced, or if a product of standard protein content is desired the higher the potential yield the greater will be the economy in manufacturing such a product. As an example, the human types examined in these studies gave three times the yield of the avian type, and assuming proportionate losses of protein in making tuberculins by heat concentration, then *apart from the question of specificity*, which need not be considered at this point, such a human tuberculin will be three times as potent as a corresponding avian product. Further, if it were desirable to produce Purified Protein Derivatives in the dry state from such growths, the yield of human P.P.D. would be three times as great as that of avian P.P.D. In considering the economics of such manufacture the yield of protein principle per unit consumption of asparagine and glycerine is an important point. Hence, for commercial purposes, it is obviously desirable to determine the yield of protein nitrogen obtainable from different strains of each type of tubercle bacillus and of *M. paratuberculosis.* Furthermore, whatever may be the relative advantages

of using reconstituted P.P.D. solutions in place of heat-concentrated tuberculins for the detection of tuberculosis in man and animals, an important factor in standardisation is a knowledge of the protein nitrogen content of all diagnostic products and hence it is necessary to have a thorough knowledge of the potentialities of the various strains of acid-fast bacilli used.

Summary

Chemical changes occurring during growth of three strains of human, one strain of avian and one of bovine type *M. tuberculosis*, two of *M. paratuberculosis* and one of *M. phlei* were studied in synthetic media of asparagine-glycerol type.

Growths corresponding to bacterial dry weight up to 1.9 g. per 100 ml. of modified Dorset and Henley medium, as used by the American Bureau of Animal Industry for preparation of tuberculin, were obtained with the human strains. Production of water-soluble, non-heat coagulable protein (the active fraction of tuberculin) was of the order of 0.15 g. per 100 ml. The principal chemical changes in the medium were rapid disappearance of dextrose and amide-nitrogen, heavy production of ammonia and extensive utilisation of glycerol. In some cases glycerol disappeared almost entirely as a result of continued bacterial respiration after cessation of growth.

Growth of the avian strain was even heavier but with a lower nitrogen content of the bacterial mass, and production of active tuberculin protein only one-third that of the human types.

Growth of the non-pathogenic *M. phlei* was very rapid, maximum bacterial weight being reached within a month. The gross chemical changes in the medium were much the same but at the point of maximum growth the non-coagulable protein fraction was only half that of the three human strains of tubercle bacilli. This figure, however, increased by a further 50-70 per cent. on standing another month, presumably by autolysis of dead cells.

Because of its lower tolerance to higher concentrations of glycerine the strain of *M. tuberculosis bovis* was grown in the modified Sauton medium of Watson and Reid. Growth was less heavy and the production of active tuberculin protein was only one-third that of the three human strains in the Dorset-Henley B.A.I. medium. Glycerol was less effectively utilised although dextrose and amide-nitrogen values in the medium diminished rapidly. Small initial ammonia production diminished with increasing growth, the final pH being on the acid side as compared with the alkaline pH of all growths on the B.A.I. medium.

Two strains of Johne's bacillus were also grown on the modified Sauton medium. The faster growing strain reached a maximum weight of 1.12 g. bacterial dry matter per 100 ml. of medium within a month, the slower requiring two months to reach 1.17 g. Production of active, non-coagulable "johnin protein" was low, reaching about 0.07 g. per 100 ml. in 10 weeks,

or less than half that of the tuberculin protein from the human tubercle bacilli in B.A.I. medium. The slower disappearance of dextrose, glycerine and ammonia nitrogen from the medium was the principal feature of the slower growing strain.

The main changes during the growth of all the organisms studied are illustrated by graphs. Since all available evidence shows that the production of soluble non-coagulable protein in the media is the factor which determines the biological activity of tuberculins, johnins and phleins, derived from bacteria-free filtrates, and the yield of Purified Protein Derivatives obtainable from such filtrates, emphasis is laid on the value of protein determinations as a means of selecting strains for diagnostic use and for estimating the potency of final diagnostic products.

Acknowledgments

Thanks are due to Mr. T. M. Doyle of this laboratory for obtaining the cultures of Johne's bacillus; to Mr. J. Reid of the Tuberculin Department for supplying broth cultures of the various organisms studied; and to Messrs. C. W. Clarke and F. J. Salt for technical assistance at some phases of the investigation.

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BOVINE TUBERCULOSIS IN THE TROPICS WITH SPECIAL REFERENCE TO UGANDA

Part II

By J. CARMICHAEL, D.Sc., M.R.C.V.S., Dip.Bact.(London)
Colonial Veterinary Service

Experimental Tuberculosis in Zebu Cattle

In Part I* of this paper attention has been drawn to the very low incidence of tuberculosis in Zebu cattle in India and Tropical Africa. This remarkable paucity of tuberculosis does not, however, apply to all cattle in

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warm climates, for in Uganda there is the Ankole breed of cattle in which tuberculosis has proved to be extremely widespread.

The environmental conditions appertaining to cattle husbandry in the tropics as compared with colder climates has on occasion been brought forward to explain these circumstances (Hornby, 1934), but, as pointed out earlier, this cannot be accepted as the true explanation. The tubercle bacillus as found in Zebu cattle has been said to be of lower virulence than normal (Sheather, 1921), but when the literature is discussed it will be seen that there is little evidence to support this contention, whilst in Uganda numerous bovine strains have been isolated from infected cattle and all have proved to be of standard virulence.

Lastly, the question of race resistance has been investigated and it appears it is here where the true explanation lies.

Experimental evidence points to the fact that Zebu cattle possess a considerably greater resistance to tubercular infection than those of European breeds in so far as standard European strains of the tubercle bacillus are concerned.

The Literature.

Liston and Soparkar (1917), referred to in Part I of this paper, concluded as the result of their experiment that the comparative infrequency of the disease among cattle in India is due to natural resistance rather than to any method of housing or keeping cattle in India as compared with England. They also point out that whereas a dose of 50 mg. of a culture of tubercle bacillus when injected into English calves invariably causes severe generalised tuberculosis, this does not apply to Indian calves, where there appears to be a much greater individual variation in resistance.

Sheather (1921) carried out inoculation experiments on buffalo and cow calves, using a local strain of tubercle bacilli isolated from a tuberculous bovine. Five buffalo calves received 10 mg. of culture subcutaneously and five received 50 mg. Twelve cow calves were similarly inoculated, half receiving 10 mg. of culture and half 50 mg. None of these animals died of the inoculation and they were killed off between 152 and 159 days later.

All animals put on weight and the post-mortem examinations were classified as follows :—

(a) Fourteen animals—

Slight tuberculosis.
A lesion at the site of inoculation.
Lesions in the thoracic glands and possibly abdominal glands.
A few lesions in the lungs or spleen.

(b) Five animals—

Extensive but not severe.

As above, but with the addition of tuberculous pleurisy or peritonitis or both.

(c) Three animals—

Generalised but not progressive. Cases in which there was evidence of invasion of the organs by way of the blood stream with the production of numerous lesions in the lungs.

The results are compared with those of the Royal Commission on Tuberculosis (2nd Interim Report, Part II Appendix, Vol. I, p. 34), which showed that a subcutaneous dose of 50 mg. of culture almost invariably produced fatal generalised tuberculosis within seven weeks and in the case of a 10 mg. dose 60 per cent. died within eight weeks.

The author concludes from his experiments both on rabbits and on cattle that the virulence of the tubercle bacillus isolated from Indian cattle is lower than that isolated from cattle in Europe. He states :—

" Glen Liston's experiments indicated that Indian cattle are less susceptible to infection with the tubercle bacillus than English animals when tested with a virus of European origin.

" The experiments here recorded indicate that this is not the sole or possibly even the most important factor in determining the comparatively frequent occurrence of tuberculosis in Indian cattle. They appear to indicate beyond all possibility of doubt that the strains of tubercle bacilli affecting cattle in India possess a distinctly lower degree of virulence than tubercle bacilli isolated from cattle in Europe.

" A point which would appear to support the view that the lower virulence of the organism is the more important factor is that in practically every instance the natural lesions which have come under observation have been restricted to a few glands and have been to a very large extent calcified."

Soparkar (1926) carried out a large-scale experiment to test the susceptibility of various Indian breeds of cattle to tuberculosis. A type strain obtained from the National Collection was used in a dose of 50 mg. inoculated subcutaneously. One hundred and forty-one calves were used in the experiment, and the results showed that whilst a considerable number (48) proved very resistant and remained well and lived beyond 90 days, the susceptibility varied considerably. Ninety-three died of acute generalised tuberculosis in periods varying from 17 to 101 days. Apparently out of these 93, only 10 showed the very severe type seen in English calves.

The author summarises the position in the following paragraph :—

" While the experiments of the Royal Commission on Tuberculosis show that a dose of 50 milligrammes of culture of bovine origin given subcutaneously is almost invariably sufficient to overcome the resistance of English (Jersey)

cattle, and, with rare exceptions, produces generalised tuberculosis of a severe type which proves rapidly fatal, the results of the present experiments would indicate that a certain proportion of Indian calves equally small in size remain well after similar infection, and, when autopsied, show slight or minimal lesions, indicating that they are possessed of very high powers of resistance to tuberculosis as compared to English calves."

Soparkar (1927), in a paper dealing with the virulence of tubercle bacilli isolated from cattle in India, reviews Sheather's (1921) results and quite rightly draws attention to the fact that Sheather used cultures which were six months old, which of course detracts considerably from the significance of his results.

Soparkar carried out inoculation experiments on rabbits and calves, using both European and Indian strains of tubercle bacilli. Of the European strains actively growing young cultures were used, also cultures which were six months old. Of the Indian strains only young cultures were used.

It is difficult to draw definite conclusions from the results, but it is quite evident that the European strains six months old were not by any means as virulent as the young cultures, either in rabbits or calves. It is unfortunate that six months old Indian strains were not used in order to make a direct comparison with Sheather's work.

Two series of calves were inoculated, one with 21-day old European cultures (Table V), and the other with Indian cultures of the same age (Table VII).

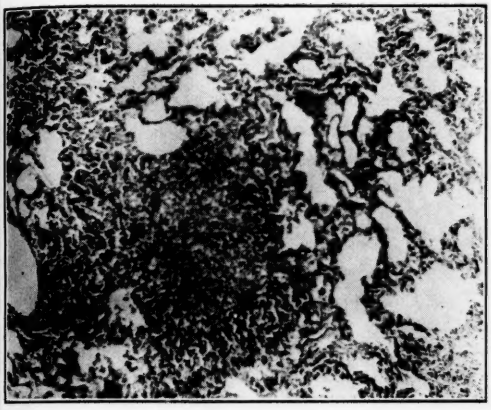
On examination of Table V, i.e., the inoculations with European cultures, 13 of the 24 calves died in an average period of 40 days, whilst Table VII, i.e., the inoculations with Indian strains, shows that 15 calves died out of 19, but the average period of survival was 71 days.

It is difficult to say on these results whether one strain is more or less virulent than the other. However, a number of calves inoculated with the Indian strain did, in fact, die of acute generalised tuberculosis in the same way as those inoculated with a known virulent strain of European origin. This is important and indicates that the Indian strain was not of markedly low virulence, and that individual differences existed in the subjects inoculated.

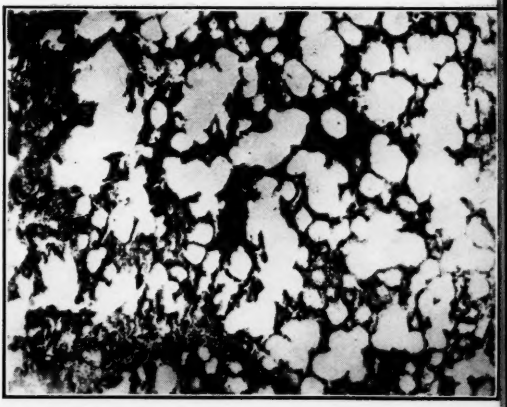
It will be recalled that none of Sheather's (1921) calves died, and when killed they only showed local lesions. All the Indian strains proved highly virulent for rabbits in the same manner as the type European strain.

Soparkar's findings are as follows: "The rarity of gross tuberculous lesions hitherto observed among indigenous cattle and the usual tendency of the lesions to remain localised in affected animals, cannot therefore be ascribed to any noteworthy difference in the virulence of the infecting organisms."

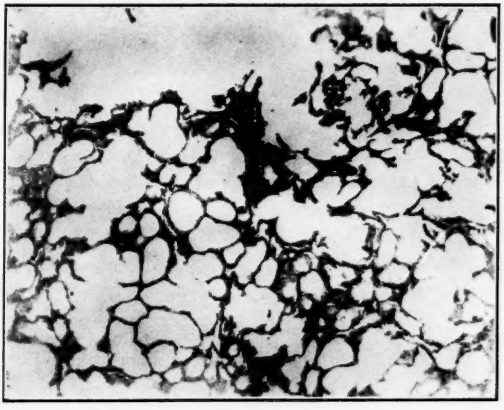
It must be remembered that the indigenous cattle of India are Zebu in type.



SECTION I.—*Control—non-resistant.* Lung of goat injected with 10 mg. of strain B.V. 34 showing severe tuberculous lesions.



SECTION II.—*Bovine* 3281—*Resistant.*—Lung 90 days after injection of 50 mg. of culture. Note general thickening of alveolar septa due to cellular infiltration but otherwise fairly clear.



SECTION III.—*Bovine* 3275—*Resistant.*—Lung of animal killed 354 days after injection of 50 mg. of culture. Note a fairly normal lung showing slight interalveolar infiltrations.

XUM

Experimental

The object of these experiments was to ascertain how Zebu calves reacted to a test dose of virulent bovine tubercle organisms with a view to discovering whether any resistance to tuberculosis did in fact exist in this breed of cattle. The opportunity was also taken to test calves of the Ankole breed for comparative purposes.

Technique.—The dose of organisms was fixed at 50 mg. (Royal Commission on Tuberculosis) and was given subcutaneously. All calves were previously tested with tuberculin by the double intradermal test and only non-reactors used in the experiment. The strains used were B.V.28 and B.V.34, kindly supplied by Dr. A. S. Griffith of Cambridge as being typical dysgonic virulent bovine cultures of recent isolation. The strains were highly virulent for rabbits in doses of 0.001 mgms. intravenously and 10 mg. subcutaneously.

The cultures were grown on inspissated ox serum and only young, vigorous cultures, not more than 10 days old were used. As controls to the actual injections of the calves, rabbits and/or goats were inoculated with appropriate doses of the same cultural growths.

It was intended to kill off all surviving inoculated calves after a period of 90 days and this was done in a number of cases, but later the survival time was allowed to extend, so as to ascertain in what manner the tissues of a resistant calf would react to a tubercle infection, and perhaps enable the degree of resistance to be more accurately assessed.

Experiment No. 1

Zebu Calf No. 3281 (weight 129 lbs.) and

Zebu Calf No. 2562 (weight 110 lbs.).

Inoculated subcutaneously with 50 mg. of 6-day-old serum grown culture BV.28.

Controls.—Two rabbits inoculated with 10 mg. of the same culture subcutaneously. One rabbit died 18 days later. Post-mortem examination showed a tuberculous abscess perforating the lumbar muscles into the peritoneal cavity. Liver, spleen and lungs full of small tubercles.

The second rabbit died after 58 days. Post-mortem examination showed acute generalised tuberculosis. Local abscess. Lungs, spleens, kidney and all glands tuberculous.

Zebu Calf No. 3281.

Killed after a period of 90 days. Condition fair. Weight same as at time of inoculation, i.e., 129 lbs. Regional gland (right prescapular) enlarged and indurated and on section contained firm, dry, caseous material tending to calcify. A soft purulent abscess in adjacent subcutaneous tissues. Presternal gland enlarged and indurated and on section showed fibro-caseous material

tending to calcify. Liver showed numerous very tiny miliary tubercles below the capsule. Hepatic gland slightly enlarged. Lungs normal in consistency but containing numerous very small translucent miliary tubercles.

A guinea pig inoculated with 0.5 c.c. of an emulsion from the prescapular gland died of severe generalised tuberculosis 106 days later.

Sections.—Lung: Interalveolar septa generally thickened and congested with occasional circumscribed consolidations composed of fibroblasts and lymphocytic cells disposed in a matrix of structureless hyaline-like material with or without a calcified centre. No giant cells present.

Liver: Very small tubercles—few in number. Epitheloid cells almost entirely replaced by a ring of lymphocytes. Centre of tubercle shows destruction of liver and in some cases complete necrosis, whilst in other cases there was an infiltration with lymphocytes and epitheloid cells. Giant cells frequent.

ZEBU CALF No. 2562.

Killed after a period of 90 days. Increase in weight 34 lbs. Condition fairly good. Regional gland (right prescapular) consisted of a large caseo-fibrous tumour-like mass. Presternal gland enlarged and gland substance entirely replaced by dry caseo-fibrous material tending to calcification. Spleen showed a few miliary tubercles scattered throughout the pulp. Liver showed numerous miliary tubercles scattered throughout the organ. Lungs showed a few discrete translucent miliary tubercles.

The impression obtained from the post-mortem examination of these two calves was that the general tendency was one of resolution and healing. There was a distinct tendency to calcification in the gross lesions in the glands whilst the miliary tubercles in the parenchyma of the organs were discrete and firm in consistency, giving the impression of sequestration and resolution.

The pulmonary tubercles in calf No. 3281, described as translucent, appeared to be undergoing a hyaline change.

Experiment No. 2

(1) ZEBU CALF No. 3279.

Received 50 mg. subcutaneously of strain B.V.28 on May 31, 1938. Killed May 20, 1939.

Duration of infection: 354 days. Weight at commencement: 132 lbs. Weight at conclusion: 284 lbs.

Post-mortem.—Condition good. Regional gland (right prescapular) about twice normal size and surrounded by a thick fibrous capsule. A tuberculous abscess about the size of a walnut well encapsulated and containing dry caseo-fibrous material is seen at the upper pole of the gland. The rest of the gland is normal, with the exception of a small encapsulated abscess about 1 c.m. diameter containing dry calcified tuberculous material. No lesions in the lungs and rest of body normal. This animal has completely recovered from the tuberculous infection and was in perfect health.

One guinea pig injected with 0.5 c.c. of emulsified prescapular gland died of generalised tuberculosis after 189 days.

Sections.—Lung: The section has all the characteristics of a normal lung. There appears to be a slight thickening of some of the interalveolar septa, and there are very occasional tiny circumscribed fibrosed areas involving perhaps six or seven *alveoli*. The fibrous tissue is laid down in a concentric manner and in the centre there may be a microscopic cavity or a small dot of calcified material.

(2) Zebu Calf No. 3277.

Received 50 mg. of strain B.V.28 on May 31, 1938. Killed January 14, 1939. Duration of infection: 228 days. Weight at commencement: 153 lbs. Weight at conclusion: 243 lbs.

Post-mortem.—Excellent condition and shows every sign of health. Regional gland (right prescapular) about three times normal size and full of creamy yellow pus forming a large tuberculous abscess. Lungs showed a few scattered millet seed-like tubercles—hard and obviously healed.

Spleen normal in size and showed old millet seed hard tubercles showing early calcification. Middle cervical gland firmly encapsulated and containing caseo-calcareous matter.

Mediastinal gland slightly enlarged and indurated. 0.5 c.c. of caseous matter from prescapular gland emulsified and injected subcutaneously into two guinea pigs which died of acute generalised tuberculosis 124 days later.

This animal showed intermittent pyrexia for the first month but gained steadily in weight.

Section.—Lung: Most of the section shows normal lung tissue. There are occasional areas where the alveoli are filled with a cellular exudate composed of epitheloid and lymphocytic cells whilst there are also occasional concentric fibrosed foci involving a few alveoli.

(3) Zebu Calf No. 3275.

Received 50 mg. of strain B.V.28 on May 31, 1938. Killed May 20, 1939. Duration of infection: 354 days. Weight at commencement: 185 lbs. Weight at conclusion: 380 lbs.

Post-mortem.—Excellent condition and appears in perfect health. The only abnormality seen was the regional gland (right prescapular), which was about twice the normal size—hæmorrhagic on section and showing an encapsulated tuberculous abscess about 1 c.m. in diameter and containing fibro-caseous material showing early calcification. Lungs, spleen and all other glands normal.

This animal had obviously recovered from the tuberculous infection and shows almost complete resistance.

It showed intermittent pyrexia for the first three weeks but gained steadily in weight.

Sections.—Lung: Lung normal except for very occasional minute circum-

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scribed fibrosed areas involving only one or two alveoli. There are also very occasional areas of circumscribed consolidation consisting of a mass of lymphocytes rather resembling a lymph node. These usually occur near a small bronchiole and occupy three or four alveoli only.

Controls.—(1) *Rabbit No.* 1071: Received 10 mg. of B.V.28 on May 31, 1938, subcutaneously and died after 92 days of severe generalised tuberculosis. *Post-mortem*: Local abscess. Tuberculosis of lungs and spleen and all glands and raised tubercles on kidneys.

(2) *Rabbit No.* 1072: Received 10 mg. of B.V.28 on May 31, 1938, subcutaneously and died after 99 days of severe generalised tuberculosis. *Post-mortem*: Local abscess. Tuberculosis of lungs and spleen and all glands. Raised tubercles on kidneys.

Experiment No. 3

ZEBU CALF No. 3274.

Received 50 mg. of strain B.V.28 on August 27, 1938. Killed May 20, 1939. Duration of infection: 266 days. Weight at commencement: 140 lbs. Weight at conclusion: 168 lbs.

Post-mortem.—Fair condition. Regional gland (right prescapular) slightly enlarged and firm and showing a few discrete encapsulated tubercles about the size of pea and tending to calcify. A subcutaneous tuberculous abscess in this region had already drained. Caseo-fibrous lesions showing early calcification were seen in the left prescapular, presternal, mediastinal, axillary (right) hepatic and left precrural glands.

Spleen showed numerous firm encapsulated tubercles 3-8 mm. diameter throughout the organ. Lungs showed many translucent firm miliary tubercles throughout. There were numerous tubercles 3-4 mm. in diameter scattered over the omentum.

The liver showed numerous firm small miliary tubercles below the capsule and throughout the organ. All showed early calcification. This animal had not resisted the infection as well as others but it had gained in weight and the tendency was to complete clinical recovery.

Sections.—Lung: This section shows rather more extensive and diffuse areas of consolidation consisting of masses of epitheloid cells in which there is evidence of early fibrosis. There is no giant cell formation. There are several circumscribed and fairly large tubercle-like formations consisting of masses of epitheloid cells through which there are well-marked concentric stands of fibrous tissue and in the centre an irregular area of calcification. Other areas are very tiny circumscribed areas of scar tissue involving only two or three alveoli with or without a tiny centre of calcified material.

Controls.—Goat No. 1106 and *Goat No.* 1107 inoculated with 10 mg. of same strain subcutaneously. Both died 39 days later and on post-mortem

showed severe miliary tuberculosis of the lungs with a local lesion in the regional gland—right prescapular.

Experiment No. 4

ZEBU CALF No. 3272.

Weight 152 lbs. Inoculated subcutaneously with 50 mg. of a six-day-old culture B.V.34 on December 22, 1939. Killed 91 days later.

Post-mortem.—Weight 175 lbs., an increase of 23 lbs. Excellent condition. Right prescapular gland (regional gland) enlarged, indurated, and full of dry caseo-fibrous material. All other glands normal. Lungs showed a very very scattered miliary tubercles with no peritubercular reaction.

Sections.—Lung: A section of a normal lung with very rare tiny areas of concentrically arranged scar tissue involving one or two alveoli and having in the centre a dot of calcified material or a small cavity, or they may be entirely solid.

Liver: Normal except for rare and very small and irregular-shaped localised infiltrations of darkly stained stellate cells. Regeneration of liver tissue appeared to be complete.

Controls.—(1) *Goat No.* 987: Inoculated subcutaneously with 10 mg. of the same culture. Died after 30 days of severe generalised tuberculosis.

(2) *Rabbit No.* 986: Inoculated subcutaneously with 10 mg. of the same culture. Died after 87 days of severe generalised tuberculosis.

Experiment No. 5

ANKOLE CALF No. 3588.

Received 50 mg. T.B. strain B.V.34 seven-day-old culture on serum subcutaneously on March 14, 1939. Killed *in extremis* April 4, 1939. Duration of infection 21 days.

Post-mortem.—Calf very emaciated. Right prescapular (regional lymph) gland enlarged and indurated and lying in a mass of fibro-caseous material. Mediastinal gland enlarged and indurated. Other glands enlarged and indurated. Lungs distended and full of miliary tubercles. Liver—numerous miliary tubercles. Kidneys—miliary tuberculosis. Spleen—miliary tuberculosis. This calf showed no resistance to infection and died of acute miliary tuberculosis.

ANKOLE CALF No. 3585.

Received 50 mg. seven-day-old culture of strain B.V.34 on serum, subcutaneously on March 14, 1939. Died after 59 days.

Post-mortem.—Emaciated. Both lungs distended and full of tubercles about 3 mm. in diameter. Marked hæmorrhagic reaction around the tubercles. Spleen swollen and on section showed numerous tubercles. Prescapular, presternal, mediastinal, mesenteric, and hepatic glands all tuberculous. Liver—dark in colour, swollen and covered with numerous tubercles

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ranging in size from a millet seed up to a diameter of 3-4 mm. These extended throughout the organ.

Controls.—(1) *Rabbit No.* 1177 : Inoculated intravenously with 0.001 mg. of same culture. Died after 41 days. *Post-mortem* : Severe generalised miliary tuberculosis.

(2) *Rabbit No.* 1178 : Inoculated subcutaneously with 10 mg. of same culture. Died after 117 days. *Post-mortem* : Severe generalised tuberculosis.

Experiment No. 6

ANKOLE CALF No. 3583.

Inoculated with 50 gm. of strain B.V.34 on March 15, 1939. Weight : 252 lbs. Died August 16, 1939. Weight : 250 lbs. Duration of infection : 154 days.

Post-mortem.—Condition poor. Lungs show numerous miliary tubercles. Liver showed miliary tubercles. Regional gland enlarged and full of caseous material. Glands generally enlarged and indurated.

ZEBU CALF No. 3582.

Inoculated with 50 mg. of strain B.V.34 on March 15, 1939. Died on October 25, 1939. Duration of infection : 194 days.

Post-mortem.—Calf in poor condition. The only sign of tuberculosis was a number of small millet seed tubercles in the spleen and an abscess in the regional gland. Lungs and liver—normal. This calf was suffering from a severe gastro-enteritis which was the probable cause of death.

Control.—Goat No. 1171 : Inoculated subcutaneously with 10 mg. of the same culture. Died after 41 days of acute miliary tuberculosis.

Discussion

The effect of the tubercle bacillus on the tissues depends on the virulence of the particular strain used and on the resistance of the host. Thus the reaction will differ with similar hosts inoculated with different strains and the same standard strain may provoke different reactions in hosts of the same species. Further, it has been observed that the reaction of a susceptible host to a strain of low virulence is similar to that of a resistant host to a fully virulent organism.

In these experiments the strain of tubercle bacillus was of a standard virulence and the object was to determine the degree of resistance of the hosts concerned—Zebu cattle—in view of the observations in Uganda which were recorded in Part I of this paper.

It is generally accepted that a bovine strain of standard virulence will kill an average calf of the European breeds in about 10 weeks when inoculated subcutaneously in a dose of 50 mg. (A. S. Griffith, 1930).

From the results obtained it will be seen that of the eight Zebu calves inoculated all showed a marked degree of resistance, whilst the three Ankole

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calves showed little resistance and reacted in a similar manner to European breeds. It would seem that in the resistant Zebu calves there was a marked generalised infection which was gradually overcome to a greater or lesser extent, depending on the degree of individual resistance, but in all cases there was sufficient resistance to preserve health, whilst in some an almost completely successful reaction had taken place.

Thus calves killed at 90 days after inoculation showed macroscopic evidence of a fairly severe infection whilst those which were allowed to survive for longer periods up to 354 days improved markedly on the earlier ones and demonstrated a progressively successful and almost complete resistance to a lethal dose of a virulent strain of tubercle bacilli.

The sections examined, although few in number, demonstrate the general histo-pathological process of recovery. Thus in the calves allowed to survive 90 days there was evidence of marked tissue reaction going on in the lungs whilst the lungs of an animal infected for 354 days were quiescent and merely showed a few scars to indicate the battle which it has successfully survived.

The rabbits and goats, as controls, amply demonstrate the virulence of the strains used in each individual inoculation. The above results have been obtained with standard European strains and work is in progress to test out locally isolated virulent bovine strains in a similar manner.

Summary

The results of inoculating subcutaneously eight Zebu calves and three Ankole calves with 50 mg. of a bovine strain of tubercle bacillus of standard virulence are recorded. The Zebu calves showed a marked resistance to tuberculous infection as compared with calves of British breeds, but the Ankole calves, on the other hand, proved equally susceptible.

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DEPRECIATION IN THE DAIRY HERD

By J. J. MacGREGOR, B.Sc., M.Sc., B.Litt.
Harper Adams Agricultural College.

DAIRY herds are subject to considerable loss through disease and other forms of depreciation. A recent report of the diseases of farm livestock (1940) (prepared by the Survey Committee of the National Veterinary Association) has estimated the loss due to mastitis, sterility, abortion and Johne's disease in dairy herds at about £20,000,000 per annum in Great Britain. A scheme based on routine veterinary inspection to reduce substantially this loss was drawn up by the Association. A charge of 12s. 6d. per cow in the herd was regarded as adequate to meet the expenses of the scheme. That this charge—small relative to potential saving—could be regarded as a useful form of insurance requires little emphasis.

The Report of the Economic Advisory Council's Committee on Cattle Diseases (Ref. Cmd. 4591, H.M.S.O., 1934) on the basis of other evidence estimated on the annual wastage of dairy cows from all causes was 22½ per cent. The following table from this Report summarises the results of three extensive surveys carried out at Cambridge, in Ayrshire and at Reading:—

TABLE I

*Causes of the Disposal of Dairy Cows**

	Summary of three Investigations
Number of herds	2,830
Number of cows in herds	78,905
Number of disposals recorded	17,247
Percentage of these disposals recorded as being on account of:	
(1) Diseases of the reproductive organs—	
(a) Sterility	23.8
(b) Abortion	3.0
Total reproductive disease	26.8
(2) Udder disease	7.7
(3) Tuberculosis, Johne's disease and wasting	9.7
(4) Death and miscellaneous diseases	10.4
(5) Tuberculin test	3.6
Total from disease	58.2
(6) Old age	4.3
(7) Accident	1.5
(8) Low yield	17.7
(9) Trade	18.3
	100.0

*Summarised from Economic Advisory Council, Committee on Cattle Diseases Report. Cmd. 4591, H.M.S.O., 1934, p. 11.

An item for "herd replacement" is a feature in any detailed costs of milk production and arises from the fact that the profitable life of the dairy cow is very short—usually about half of the theoretically useful span of nine years. Cows are normally purchased into or transferred into herds at or near the most productive stage of their lives and consequently when sold several years later have been considerably reduced in value. This is to be expected even when the cows are sold in good health but deaths and accidents also take their toll and the "herd replacement" item can be a significant part of the total cost of milk production. For instance, in the year ending September 30, 1939, an analysis of the cost of milk production per gallon for 382 wholesale, 123 graded and 47 producer retailer herds in England and Wales gave the following information :—

TABLE II

Cost of Milk Production, 1938-1939*

(Per Gallon d.)

	Wholesale	*Graded*	*Producer Retailer*
Foods	5.95	6.65	6.17
Labour	2.18	2.51	2.90
Miscellaneous	1.24	1.68	1.52
Herd replacement	.98	.88	1.35
Gross farm cost	10.35	11.72	11.94
Credits	.86	.98	.89
Net farm costs	9.49	10.74	11.05
	gals.	gals.	gals.
Average total yield	646	624	672
Average total net cost per cow ...	£25 10s. 10d.	£27 18s. 6d.	£30 18s. 9d.

The above table shows that "herd replacement" costs were at least 10 per cent. of the total net cost on the farm or about 50s. to 60s. per cow. This, however, does not give a real measure of the indirect loss which is brought about by loss of potential yield of milk, calves, etc. The immense reduction, apart from loss of quality of milk, which is brought about by ordinary outbreaks on farms of mastitis and contagious abortion needs no emphasis here. It will be appreciated in these circumstances that the National Veterinary Medical Association's Scheme promises to be a very satisfactory and economic form of insurance.

* Summarised from the "Cost of Milk Production in England and Wales," Survey of Interim Report No. 5, Milk Investigation Scheme. Issued by the Agricultural Economics Research Institute, Oxford, 1940, pp. 6-7.

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The following article attempts to relate the value of cows purchased or transferred into the herd to the returns received for cows disposed of for different reasons. The data were obtained from the West Midland part of the Milk Investigation Survey (i.e., Shropshire, Staffordshire and Warwickshire) and relate to the year ending September 30, 1940.

TABLE III

Causes of Disposal and Values of Cows in Herd, Purchased and Sold. West Midland Province, 1939-1940

Number of herds		48
	No.	*Average Value.*
Cows in herd: Opening valuation	1925	£19 5 9
Closing valuation	1966	20 6 10
Average in herd	1945	19 16 7
Disposals recorded	620	18 2 9
Purchases recorded	322	23 15 7
Transfers in	345	22 16 2

It is necessary to state that with the scheme running for a number of years the practice has been to keep the valuations as conservative as possible to avoid any "paper" values influencing the costs too substantially. The normal practice has been to value purchased cows at or near their purchased price and to maintain the others cows in the herd near their previous values. Home-bred cattle transferred into the herd were also maintained at conservative values and in 1939-1940 were generally valued at considerably less than their market value. In the conclusions which are made here these qualifications are important, since any writing down in the valuation will tend to reduce the amount of herd depreciation brought about by disposal of cows at levels below the purchased prices.

As was to be expected, the purchase price of cows is above the average value of all cows in a herd. Disposals represented 31.81 per cent. of the average number of cows in the herd—a proportion which is above that recorded by the Report of the Economic Advisory Council. The drop in value between purchases and sale was about £6. As all cows disposed, however, are not diseased, the reduction in value through disease cannot be accurately estimated from the average figures.

Unfortunately, no reason was given for the disposal of 98 out of the 620 cows included in the above average, but on the remainder it is possible to obtain the average price of cows disposed of for definite reasons.

The following table summarises the information as far as possible by convenient categories of disposal :—

TABLE IV

Reasons for Disposal

		Per cent.	*No.*	*Average Price* £ s. d.
1.	(a) Sterility, etc.	22.58	140	18 6 5
	(b) Abortion	.48	3	20 15 10
	Total	23.06	143	18 7 0
2.	Udder disease	4.68	29	17 0 8
3.	T.B., Johne's disease and wasting ...	4.68	29	4 10 7
4.	Deaths and miscellaneous diseases ...	5.65	35	1 13 11
5.	T.T. reactors	5.16	32	21 11 5
6.	Old age	2.26	14	15 6 8
7.	Accidents	2.58	16	2 13 8
8.	Low yield	4.35	27	23 14 7
9.	Trade	8.87	55	27 18 8
10.	Fat	20.97	130	20 4 1
11.	Miscellaneous	17.74	110	19 16 6
	Total	100.00	620	£18 2 9

For several reasons it is not possible to analyse all the evidence on the same basis as in the first table quoted. For one reason a very large number of cows are entered as " fat " and presumably this will include a large number of cows which could be classified as barren or having suffered from abortion. Only three cows were definitely described as being disposed of because of abortion.

Deaths in the dairy herd due to diseases were 15.01 per cent. of the total causes. A further number of deaths due to accidents have been directly entered in category 7 of the above table, although a number of accidents did not prove fatal.

Analysis of deaths due to disease indicates that the most common causes were: Milk fever, three; complications when calving, eight; and tuberculosis,

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two; as shown in the following table. The return on dead cows is, of course, very low.

TABLE V

Deaths in Dairy Herds

West Midland Province, 1939-1940

	Shropshire				*Staffordshire*				*Warwickshire*			
		Value				*Value*				*Value*		
	No.	£	*s.*	*d.*	*No.*	£	*s.*	*d.*	*No.*	£	*s.*	*d.*
Abscess on lung ...	1	1	0	0	—		—		—		—	
Complications when calving	5	6	7	6	1	1	0	0	2		—	
Unknown	1	0	10	0	—		—		—		—	
Milk fever	1	2	0	0	2	0	10	0	—		—	
Wooden tongue ...	1	1	0	0	—		—		—		—	
Rupture	1	0	15	0	—		—		—		—	
No reason given ...	2	1	0	0	2	1	0	0	3	5	10	0
Accident	1	0	8	0	3	1	0	0	3	1	10	0
Inflammation of stomach	1	1	0	0	—		—		—		—	
Poisoning (?)												
Anthrax	—		—		1		—		—		—	
Garget	—		—		1	0	15	0	—		—	
Blizzard	—		—		2	1	10	0	—		—	
Mastitis	—		—		1	0	10	0	—		—	
Waster	—		—		1	1	0	0	1	0	10	0
Drowned	—		—		—		—		4	1	10	0
Tuberculosis ...	—		—		—		—		2	1	10	0
Womb infection ...	—		—		—		—		1	10	0	0
Poisoned	—		—		—		—		1	20	0	0

Clinical Articles

A CASE OF AN UNUSUAL SKIN COMPLAINT AND SUDDEN DEATH

By O. B. WOODWARD
Burton-on-Trent.

Subject: An Eight-year-old Bull-Mastiff Dog.

This dog had been treated for acute moist eczema with calamine cream by the kennelman for about a month, and then, as one patch healed and others broke out, I was called in. I found that the dog had a large bare black patch the size of the palm of the hand on the left hind quarter, but this was dry, and eventually the hair grew. On the cheek, elbow and hock of each side were areas of moist, black skin, denuded of hair, which, when squeezed, exuded pus and blood over their entire area.

I suspected Follicular Mange and on making a microscopical examination of the discharge found numerous Demodex Folliculorum present. I gave the kennelman a sulphur preparation and he did the necessary dressings. After a month the condition was worse and the dog was admitted to the infirmary. A course of arsenical injections was given and sulphur dressings were applied to the skin for two weeks with no improvement, so it was decided to open into the discharging cheek. On doing so the skin was found to be thickened and loose hair was lying in pockets in the subcutaneous tissue. I decided the only cure was to open into every part of the skin from which pus or blood could be squeezed, clean it out, remove all the hair and then dress the wound with sulphonamide ointment. After two weeks of this incising and cleaning out of cavities on both cheeks, elbows and hocks, the trouble was almost cured and there were no signs of further outbreaks; hair was growing in some parts and the bald patches were reduced in size. The dog had improved in his general health and looked very well.

He went out for his exercise, where he appeared in good spirits, came back, ate some food, was groomed and was left in an enclosed run. Half an hour later he was found leaning against a wall, slobbering, his sides greatly distended and looking very distressed. Fearing he had picked up poison in the fields he was immediately given emetics, which had no effect, and by this time the distension was intense; copious enemata gave no relief and the stomach

tube apparently doubled back. Fearing rupture of the stomach, it was punctured with a fine trochar and canula, and by this the distension was very considerably relieved, but in a few minutes it was as intense as ever.

The dog had lost consciousness in an hour from the onset of symptoms and died in six hours.

On post-mortem examination the stomach was found to be enormously distended, occupying almost the whole of the abdominal cavity; it was intensely congested and twisted, a half turn on the œsophagus. From this it appeared that the twist at the œsophageal opening had prevented any vomiting and the pylorus, being pushed over, was completely closed, so there was no possibility of any escape of gases into the intestines and hence the failure to effect any relief.

There appeared no cause whatever for this sudden and fatal illness.

It has been suggested that this was primarily a case of so-called "Great Dane Disease," i.e., acute distension of the stomach, and that the torsion was secondary to this. It is the first case of either this skin condition or this form of sudden death that I have seen in fifteen years of canine practice, and I would be interested to hear of any other similar cases.

CANCER OF THE RECTUM IN A DACHSHUND

By O. B. WOODWARD

Burton-on-Trent.

Subject: A Black and Tan Dachshund Dog, six years old.

Advice was sought by telephone for the treatment of this dog. A condition of enteritis with occasional slight hæmorrhage was described and astringent medicine sent. Improvement was reported in a few days and then in a couple of weeks the trouble returned and more medicine was provided.

Two weeks later the dog was brought into the surgery to be kept under observation. The owner now described the dog as constantly straining and passing small quantities of bloodstained fæces which, if formed, were only about the size of the bore of a lead pencil. The dog was in good condition.

On rectal examination a thickening on the wall of the rectum and about an inch in from the anus could be felt. It was raised up and about the size of a shilling.

After a consultation with the owner, and considering the good bodily condition of the dog and its comparative youth, an operation was decided upon.

Under a general anæsthetic the rectum was everted and the double thick-

ness of bowel sutured anterior to the affected part, which was then amputated, about one and a half inches of bowel being removed. Next day the dog was running about and eating well. There was a lot of straining but no hæmorrhage and steady improvement was noted each day.

A fortnight later he went home and his owner has reported how well he seems. There is a little straining after defæcation but not so severe as previously. When he went home there was a slight cicatricial constriction in the rectum.

The piece of bowel containing the growth was sent for pathological examination and was reported as an adeno-carcinoma.

Correspondence

To the Editor of THE VETERINARY JOURNAL.

Sir,—On looking through THE VETERINARY JOURNAL for September I find the abbreviation " Vet." no fewer than sixteen times. If such a contraction must be insisted upon for the economisation of printing space, why not " Vet'y "?

Personally, I would like to see the word " Veterinary " eliminated from our vernacular altogether, and be replaced by Comparative Medicine and Surgery.

And, while on this subject, may I ask is it not long past time for a thorough overhaul of our nomenclature? For example, such crude words as poll evil, quarter ill, blackhead, etc., should be taboo.

Such titles are neither artistic nor scientific, and ill becomes a profession calling itself learned.

Truly yours,
W. M. SCOTT.

Fryern Hall, Bridgwater.
November 4, 1941.

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